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# Chromatin-reduction and Tetrad-formation in Pteridophytes.

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(Plates 295, 296.)

From the time when Van Beneden, in 1883, found that mature reproductive cells have only half as many chromosomes as the ordinary somatic cells, until the present time, cytologists have endeavored to explain the meaning of this reduction and to show how it takes place. These efforts have not as yet been attended with complete success.

One of the most widely known theories as to the meaning of reduction is that of Weismann who accepted the earlier conception of Roux (1883) as to the significance of mitosis and built upon it an elaborate theory of development. In this he predicted that a form of mitosis would be found in the maturation of the reproductive elements "in which the primary equatorial loops are not split longitudinally" (p. 371) and "by means of which each daughter nucleus receives only half the number of ancestral germ-plasms possessed by the mother nucleus" (p. 375).

Weismann's prediction has been confirmed by recent observations on the copepod crustacea, and it is now known that in this group of animals at least, the chromosomes of a maturing cell undergo a transverse division, giving reduction in the Weismann sense. This process of reduction, wherever definitely made out, is invariably preceded by an arrangement of the chromatin into four-parted chromosomes, to which the name "*Vierergruppen*" or "tetrads" has been given. These tetrads are always half as numerous as the chromosomes of the somatic cells and often differ widely from them both in shape and size.

The entire question of reduction rests upon the manner of tetrad-formation, but unfortunately, observers are diametrically opposed in their descriptions of the process. On the one hand it has been shown beyond question, that in some cases (the copepod crustacea) two of the four parts of the tetrad are formed by longitudinal division of the spireme-segment, while the other two arise by transverse division. In such cases two successive mitoses divide the tetrads, first into two dyads and second into single elements. By these two divisions the resultant reproductive cell receives one-fourth of each of the original tetrads. On the other hand, in another case, *Ascaris megalocephala*, where the facts also seem to be beyond contradiction, Brauer has shown that the tetrads arise by double longitudinal division of the spireme-segments and that no transverse division takes place. In this case reduction is purely quantitative and not qualitative.

The botanists Guignard (1891) and Strasburger (1888) have maintained that in plants also, a reduction in the Weismann sense does not take place. Neither Guignard nor Strasburger found tetrads. They described the spireme as breaking up into half the normal number of chromosomes which undergo simple longitudinal division at each successive mitosis.

It would be remarkable if a process so general in animal cells as the formation of tetrads should not be found in plant cells, and with the hope of finding some evidence of this in plants I undertook the study of reduction in the group of Pteridophytes, the results of which are given in the following section.

## I. OBSERVATIONS.

Guignard and Strasburger found that in *Lilium* and in *Allium* the pollen-grain after reduction, undergoes subsequent mitoses, in each of which the same reduced number of chromosomes is retained. This led Overton (1892) to suggest that reduction in the higher cryptogams, where sexual and asexual generations alternate, might take place as far back as the formation of the spore. He also suggested that all of the cells of the sexual generation might have the reduced number of chromosomes, and in 1893 he strengthened his position by showing that the cells of the endosperm in the Gymnosperm *Ceratozamia*, divide with the reduced number. But it was Strasburger (1894) who proved it, beyond doubt, by showing that in the fern *Osmunda* all the cells of the prothallus have the reduced number of chromosomes; in other words that all cells derived from the spore have half as many chromosomes as the cells of the asexual generation. Reduction in ferns, therefore, takes place during the formation of the spore and here, if anywhere, we must look for the tetrads.

*A. Material and Fixation.*—Two fern species (*Pteris tremula* and *Adiantum cuneatum*) were selected, which were found in a fernery in October, to be in various stages of spore-formation. The pinnae were removed and cut into small pieces before fixation. The fixing agents used were 1, Herman's platino-aceto-osmic mixture; 2, Von Rath's fluid; 3, corrosive sublimate; 4, sublimate-acetic; and 5, picro-acetic. The best results were obtained with 1, 3 and 4. The pieces were imbedded in paraffine and cut horizontally in sections about 4 to 5 $\mu$ thick. The stains used were Heidenhain's haematoxylin, either alone or with orange G or Congo red as counter stains in the majority of preparations, although Flemming's triple stain gave good results.

*B. Terminology.*—In animals three periods are recognized in the development of the reproductive cells (O. Hertwig). 1. A "division period," during which the primordial germ cells ("oögonia" and "spermatogonia") increase by ordinary mitoses; 2, a "growth-period," during which the primordial germ cells enlarge and during which tetrads are formed. These cells are known as "primary oöcytes" and "primary spermatocytes," and 3, the "maturation-period," during which the nuclei with tetrads are divided by mitosis into nuclei with dyads ("secondary oöcytes" and "secondary spermatocytes"). These are again divided to form eggs or spermatids.

As regards the periods of maturation it can now be shown that both male and female cells in many animals, and reproductive cells in some plants (Pteridophytes), conform to the same type. It is well known that in spore formation of *Pteris*, the single primordial cell ultimately gives rise to sixty-four spores. It is also known that there are sixteen so-called "spore-mother-cells" in each sporangium, and that from these sixteen cells the final spores are formed. The stage between the first spore cell and the sixteen cells is directly comparable with the "division-period" in animal maturation. The sixteen-cell stage (see next page) is the stage of enlargement and of tetrad-formation and corresponds with the "growth-period." Finally the "maturation-period" is recognized by the two consecutive divisions of the sixteen cells to form the sixty-four spores.

As the maturation phenomena are so closely parallel in plants and animals, it would be of advantage to have the corresponding cells designated by analogous names. In some cases the present botanical names are unwieldy and inappropriate. The "spore-mother-cell" as a designation for the cells of the sixteen-cell stage is incorrect as well as clumsy; for, if we consider the nuclei alone, these cells do not give rise to the spores, but to the "mother-cells" of the spores, and are therefore the "grandmother-cells" of the spores. I propose, therefore, to use the term "primary sporocyte" for the cells of the sixteen-cell stage, and "secondary sporocyte" for cells of the thirty-two-cell stage. These terms are directly analogous to "primary" and "secondary" "oöcyte," and "spermatocyte" in animal cells. The term "sporogonium," which in this connection would be analogous to the term "oögonium" or "spermatogonium" is, unfortunately, already used in a different sense in botanical nomenclature and I shall therefore designate the first eight cells in spore formation as "archesporial cells" in accordance with botanical usage.

*C. Observations.*

It is beyond the scope of this paper to describe in detail the formation of the sporangium, with its annulus and tapetum, or to describe the degeneration of the tapetal cells. Nor is it necessary to give aminute account of the mitoses leading up to the formation of the sixteen sporocytes. They all agree with the division of the "archesporium" and the number of the chromosomes apparently remains the same, although they are so numerous that it is impossible to give the absolute number. From careful counting in several cases, I estimate the number to be between one hundred and twenty and one hundred and thirty.

The unicellular archesporium, which is destined to give rise to all the spores of the sporangium, can be readily distinguished from the surrounding somatic cells. It is larger, and the chromatin of the nucleus stains much more deeply than that of the other cells. The chromosomes are large and distinctly looped, and in the metaphase of karyokinesis they are split by a longitudinal division (Fig. 17). After each division the daughter-nuclei pass into the resting stage, during which the cell-walls are completely formed and each daughter-cell becomes completely separated. The resting stages are comparatively long and the division stages short.

1. *The growth-period.*

After the sixteen primary sporocytes are formed the nuclei pass as usual into the resting stage (Fig. 1). The nuclei are at first comparatively small (10.3 $\mu$ in diameter), the chromatin-reticulum does not stain intensely, and there are usually from one to three or more nucleoli in each. Meantime the tapetal cells degenerate, giving room for growth of the reproductive elements. This growth must begin very soon for the cells in the resting stage (Fig. 1) are not frequently found. When fully grown, the nuclei measure about 14.5 $\mu$ in diameter, an increase of nearly 50 per cent. During this enlargement the chromatin reticulum is converted into a delicate moniliform spireme. This is a single thread of chromatin, very much coiled and interwoven and at first distributed evenly throughout the nucleus (Fig. 2). From this condition of extreme delicacy and expansion the chromatin soon passes into a stage of greater localization and the spireme becomes thicker. Evidence of the beginning of concentration can be seen in Fig. 2 an early stage, where the nucleolus has not disappeared. In a later stage, to which Farmer has given the name "Synapsis," the meshes are drawn towards one side of the nucleus into a much more compact chromatin mass (Fig. 3). This mass next becomes loosened and the filaments more or less isolated. In exceptionally favorable preparations the spireme in this stage is seen to be double (Fig. 3 x).

The concentration of the chromatin at the same time with the thickening of the spireme seems to indicate a coalescence and union of the formerly distinct granules of chromatin in the delicate moniliform spireme.

2. *Period of tetrad formation; pseudo-reduction.*

In the case of animals when the spireme thread breaks up into segments destined to form tetrads, the number of these segments is, in general, half the number of chromosomes in the somatic cells. There is a reduction in number of chromatin masses, but the nucleus still contains all the chromatin it held at first, so that actual reduction has not yet taken place. Rückert (1894) has accordingly proposed the expressive term "pseudo-reduction" for this preliminary halving of the number of chromosomes.

*Pteris* forms no exception to this rule. The double spireme breaks up into short and well defined chromatin segments (Fig. 5 a) each of which gives rise to a tetrad. The number of these segments is difficult to determine; in several cases I counted about sixty. This is about half of the number in somatic cells where, as nearly as I can make out, there are between one hundred and twenty and one hundred and thirty chromosomes. It is an interesting fact that the process of tetrad formation is subject to some variation and does not, apparently, conform exclusively to any one type. This conclusion is based upon the following facts. The spireme segments are, from the beginning, invariably double (Fig. 5 a). The same nuclei contain various modifications of the double segment. Some of them are split in the center while the ends remain connected, giving rise to ring forms (Figs. 4, 5, 19 c). In some there is no separation at all, in others the ends separate, giving rise to "cross" forms (Fig. 6 l and Fig. 19 a) and in still others one half the segment may slide along on the other half till the ends are no longer contiguous (Fig. 6 d and e). There may be still further modifications of the double segment in the same nucleus (Fig. 5 x). In none of the nuclei which I have examined does any of these types predominate; and from their various and diverse shapes it is impossible to regard them as developmental stages of a single type. I am forced, therefore, to the conclusion that, in these ferns, tetrads may be formed in a variety of ways. The various methods can be grouped into three types, which I will describe separately as (a) the "ring type;" (b) the "rod type;" and (c) the "cross type."

*a. The "ring type."* Almost every primary sporocyte contains from one to several (8 or 9) ring forms in different stages. In some cases the chromatin portion is thin and the opening comparatively large (Fig. 4 c, 5 c, 19 c). In no case is the ring thin and delicate as in *Heterocope robusta* (Rückert, 1893, Fig. 23). The ring stage begins with a lateral bulging of the two halves of the spireme segment (Fig. 6 i); this is followed by the appearance of a furrow at the center. This furrow enlarges until it forms a circular space, and, the ends of the segment remaining attached, the chromatin forms a closed ring (Fig. 19 c). The chromatin then begins to accumulate in four parts, each half of the originally double spireme forming two (Figs. 6 J, 19 c and 20 c). These parts become more and more distinct and individualized; more compact and tightly packed together, until finally the tetrad is completed (Fig. 7). The tetrad is, therefore, derived first, by a longitudinal splitting of the spireme segment, and second, by the transverse splitting of the two halves.

*b. The "rod type."* The tetrad begins as before with the short and somewhat thickened double spireme-segment, but here no separation of the two parts of the segment takes place (Fig. 5 a). The chromatin segregates at the two ends in four swellings (Fig. 6 b). These swellings enlarge, become more definite and the segments become shorter by the gradual drawing together of the ends. The ends finally round out and tetrads are formed by what would seem to be the simplest method possible.

There are some modifications of this type. In some cases the two halves of the spireme segment slide along on each other until, in an extreme case, the opposite ends may become contiguous (Fig. 6 d, e). The resultant tetrad does not differ essentially from one formed in the simpler manner. There is the same segregation of chromatin at the four ends, the same shortening of the segment and finally the same end result, although at first the tetrad is somewhat distorted. In the rod type, therefore, the tetrad originates first by a longitudinal division of the spireme, and second, by transverse division of the halves and is equivalent in all respects to the tetrad of the "ring type."

*c. The "cross type."* In this type the halves of the double spireme segment, instead of separating in the centre as in the "ring type," or of remaining parallel to each other as in the "rod type," become separate at the two ends but remain attached to each other in the centre. (Fig. 6 a, l., etc.) These ends separate farther and farther until each half segment forms a loop which lies in contact with the other half segment only at the center of the convex side (Fig. 6 c). It is the opposite of the ring type. In some cases the segregation of the chromatin begins at an early period (Fig. 6 a), and, as separation continues, the segregation becomes more marked, until finally there are four distinct swellings lying at right angles to each other (Fig. 6 c, 19 a). The loops meantime become shorter and shorter, until finally the four parts of the chromosome are brought together, and a tetrad is formed similar in all respects to those of the "ring" and "rod" types (Fig. 6 k).

Like the "rod type," the "cross type" shows some modifications. After the ends have begun to diverge as in the normal cross type, one of the loops may swing around through an angle of 90 degrees on the point of attachment as a pivot (Fig. 6 f). It thus comes to lie in a plane at right angles to its original position. Segregation of the chromatin gives rise to the four parts of the chromosome as before. Various other modifications of this type are found (Fig. 5 x), but in all of them the result is the same. Here, therefore, as in the other types the tetrad originates first by a longitudinal division of the spireme-segment and second by transverse division of the halves.

3. *Period of Reduction.*

It is in this period of spore development that reduction of the chromosomes actually takes place. It begins with the arrangement of the mature tetrads into the nuclear plate of the primary sporocyte spindle. Before this arrangement the tetrads are distributed throughout the nucleus (Fig. 7). The nuclear membrane disappears, and after this, for the first time, it can be clearly seen that the nuclear space is filled with almost parallel spindle fibres (Fig. 8). The latter at this stage could not be traced to definite points at the poles. The tetrads lie in various positions on the spindle fibres (Fig. 9), but they gradually collect at the equator of the spindle. The migration towards the equator of the spindle is clearly shown in Fig. 10 for *Pteris* and Fig. 20 for *Adiantum*, while Fig. 11 shows the completion of the spindle in *Pteris* and the definite formation of the nuclear plate. In this stage the tetrads are closely packed, and are so numerous that counting is impossible. In the early stages, however (Figs. 8 and 10), it can be seen that the number is about sixty.

The compact arrangement of the tetrads in the nuclear plate leaves no chance for orientation. It is impossible, therefore, to tell from this division whether the tetrad divides through the line of original cleavage, or through the secondarily acquired transverse cleavage. In other words, it is impossible to tell whether the division of the primary sporocyte is a reducing or an equation division. There is, however, good reason to regard this as an equation division, and the division of the secondary sporocyte as transverse, and, therefore, as a reducing division. The second mitosis follows closely on the first, but in the short interval the two parts of each dyad, which at first appear like two small balls closely pressed together (Figs. 12 and 21), now become drawn out in the direction of their common axis, which is probably the original longitudinal axis of the spireme-segment (Figs. 13 and 14). It is immaterial in the final spore cells whether the first or the second division is a reducing division in the Weismann sense. That one of them must be is shown by the method of tetrad formation; but, from the manner in which the dyads elongate, the probability is certainly strong that reduction is effected by the second mitosis. The change in shape of the chromosomes in the secondary sporocyte-spindle makes the general appearance of the nuclear plate conform more nearly with that of the somatic cells (cf. Figs. 13 and 18), although they are fundamentally different.

4. *The spore.*

The cylindrical shape of the daughter chromosomes as they come from the division of the dyads in the secondary sporocytes is retained until late in the anaphase (Fig. 14). The resulting four daughter-nuclei lie freely in a single cell which, until the cell-plates are formed, is a syncytium. In the division of multinuclear cells it has been frequently noted that the nuclei are connected by spindle fibres. This occurs in ferns, and long after division, and even as late as the telophase after the cell-plates are formed and the nuclei have gone into the resting stage, fibres can still be seen connecting each nucleus with all the others (Figs. 14 and 15). While the cell-plates are forming, the chromosomes gradually disintegrate and pass into the reticulum although their outlines can be dimly made out even after the reticulum is well formed and the nucleoli have reappeared (Fig. 16).

5. *The centrosome.*

It is extremely difficult to stain, and correspondingly hard to find the centrosomes in this material; even at the spindle-poles its identification is not easy. I was able, however, to make it out in two different stages (Figs. 11, 12 and 13). One of these was in the mitosis of the primary sporocyte, the other in that of the secondary sporocyte. In the first of these the centrosome at the spindle-poles was double (Figs. 11 and 12), in the second it was single (Fig. 13).

## II. TETRAD-FORMATION.

In cases where reduction in the Weismann sense is actually known to take place, there have been wide variations in the accounts of the process. It was first described by a former pupil of Weismann's, Ishikawa, who did not find tetrads and who held that reduction in the copepod *Diaptomus* is accomplished by the separation of entire chromosomes. This result is entirely contradictory to the more recent results obtained by the subsequent study of *Diaptomus* and other copepods.

Vom Rath, apparently the first to correctly interpret the formation of tetrads, gave a different account of reduction in the insect *Gryllotalpa*. He found that the spireme is double before it breaks up and that there are half as many of the double segments as there are chromosomes in the somatic cells. The halves of the double segment separate, except at the ends, and a ring is formed. Later the tetrads arise by concentration of the chromatin at four points of the ring, a method by which the four parts of the tetrad originate by longitudinal division represented by the original division of the spireme, and by a transverse division.

Häcker gave still another description of tetrad-formation in *Cyclops strenuus*. His details have been denied by Rückert, who, however, accepted the general results and agreed with him that the tetrad is formed as in *Gryllotalpa* by a longitudinal and a transverse division of the original spireme segments. Rückert (1893 and 1894) has found two modes of tetrad formation, each giving, however, the same results, viz.: the formation of tetrads by a primary longitudinal and a secondary transverse division of the spireme. The first method (*Cyclops*, *Canthocamptus*) agrees almost exactly with what I have here described as the "rod type." The spireme is divided longitudinally before segmentation into half the normal number of chromosomes. The chromatin then begins to collect into a much thicker double rod; the rod then divides transversely and the tetrad is formed by longitudinal and transverse division. The other method described by Rückert takes place in *Heterocope* and *Diaptomus* and agrees very closely with what I have described as the "ring type." The double spireme breaks as before, but the double segment, instead of remaining contiguous throughout, becomes separated in the middle, while the ends alone remain in contact. A ring is thus formed and tetrads arise later by two divisions, one through the diameter of original cleavage, the other at right angles to this line.

Enough has been given in this account to show that certain methods tetrad-formation are characteristic of certain species of animals. Up to the present time it has always been found that the tetrads in a single nucleus are formed by one method, either by the "rod type" or by the "ring type" alone. My observations on the fern, however, show that tetrads in the same nucleus may be formed by both methods or even by a third. From these facts the conclusion seems inevitable that all the types of tetrad-formation mentioned above are merely modifications of the same process and have no significance in themselves so long as a common result is obtained.

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It seems remarkable that such obvious structures as tetrads should have been hitherto overlooked in the plant reproductive cells. Many observers have noticed that the mitosis in the sporocyte differs from that of all other cells, whether somatic or archesporial. This difference was early recognized by Guignard and Strasburger. Overton and Belajeff also were struck by the peculiar shape and appearance of the chromosomes in this mitosis; the latter especially described them as agreeing in all particulars with Flemming's account of heterotypical mitosis. Finally Farmer (1895), in speaking of the pollen-mother-cell of *Lilium Martagon*, refers to structures which can be explained only as early stages in tetrad formation: "Die Form der Chromosomen ist sehr unregelmässig, zuweilen erscheinen sie als Bänder, oft als Ringe mit einer oder zwei Protuberanzen, letzteres tritt namentlich in etwas späteren Stadien auf. Ich habe viele Zeit geopfert, mit dem Versuch zu einer festen Entscheidung darüber zu kommen, ob die Ringähnliche Form wirklich primitiv vorhanden oder einer inneren Spaltung zuzuschreiben ist, die das Chromosom noch nicht vollständig getheilt hat. Ich neige stark zu letzterer Annahme und betrachte desshald die Ringform, wo sie verkommt als ein frühes Anzeichen der Langstheilung des Chromosoms" (p. 58). Again he says: "Es braucht kaum bemerkt zu werden, dass diese heterotype Form der Mitose auf die Pollenmutterzellentheilungen sich beschränkt und sich weder in den vegetativen, noch in den früheren Archesporialen Theilungen derselben Pflanze findet" (p. 64). And finally: "Was die zweite Kerntheilung in Pollenmutterkorn betrifft, so zeigt sie gar nichts von den eigenthümlichen (heterotypischen) Vorgänge, welche die erste Mitose characterisiren, sie weicht nur durch die behaltene reducirte Chromosomenzahl von einer vegetativen oder eine frühen Archesporialen Kerntheilung ab. Es ist daher wahrscheinlich, dass die der ersten Theilung besonderen eigenthumlichkeiten mit der plötzlichen Chromosomenzahlveränderung in einer directen und causalen Beziehung stehen" (p. 67).

The still later work of Miss Sargant ('95), on the chromosome of the pollen-mother-cell of *Lilium Martagon*, shows that a transverse division of the chromosomes probably takes place. She does not mention the formation of rings which were described by Farmer as preceding the nuclear plate stage, nor does she mention tetrads. But as Wilson first pointed out ('96 p. 197), her description of the dividing chromosomes give strong reason to believe that these structures are to be interpreted as tetrads.

The fact that so many observers have described phases which suggest more or less clearly the formation of tetrads in different groups of plants, together with my own observations on the ferns, render it probable that further study will show the reduction of chromosomes through tetrad formation to be a phenomenon of as wide occurrence among plants as it is already known to be among animals.

## SUMMARY.

1. The spore maturation in Pteridophytes agrees step by step with the maturation of sexual cells in animals.

2. As in animal maturation, the process of spore-formation can be divided into three periods of division, growth and maturation. The *division-period* is the interval between the archesporium and the sixteen-cell stage of the sporangium. The *growth-period* is the interval during which the sixteen cells enlarge and tetrads are formed. The *maturation-period* includes the two successive divisions of the nuclei in the sixteen-cell stage, and the formation of the spores.

3. Different terms are used to designate the cells in the different stages of maturation. Those of the "division-period" are known as the "*archesporial cells*." Those of the "growth-period" have hitherto been known as the "spore-mother-cells." The term "spore-mother-cell" is, however, inaccurate and clumsy, and I suggest the term *primary sporocyte* in its place, also *secondary sporocyte* for the daughter-cells of the primary sporocyte. These are the mother-cells of the spores and by their subsequent division the sixty-four spores are formed.

4. The "growth-period" is the most important stage in maturation. It begins with a distinct enlargement of the cell. The chromatin then forms a delicate moniliform spireme before the nucleolus has disappeared. A much thicker spireme is subsequently formed from the moniliform thread. The thickened spireme then splits longitudinally. It next breaks up into half as many double spireme-segments as there are chromosomes in the somatic cells; each of these double elements forms a tetrad.

5. Three types of tetrad-formation are found in each nucleus. These may be called the "rod type," the "ring type" and the "cross type." In the first type the halves of the double spireme segment are completely separated; in the second, the halves become separated in the center but remain connected at the ends; in the third type the halves become separated at the ends but remain connected at the centre.

6. In all three types the tetrads are finally formed by a transverse division of the halves of the double spireme-segment, giving reduction in the Weismann sense.

7. These methods of tetrad-formation have no significance in themselves so long as a common result is obtained. They may be considered as modifications of the same process.

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## Description of Plates 295, 296.

Figures 1–18 are stages in *Pteris*; figures 19–21 are *Adiantum*; all figures are drawn with a Zeiss Camera lucida.

Fig. 1. Early sporocytes in the resting stage subsequent to the last division of the archesporial cells. × 1800.

Fig. 2. Primary sporocyte after growth. Chromatin arranged in a delicate, single, moniliform spireme, nucleolus persistent. × 1800.

Fig. 3. Primary sporocyte with thickened and concentrated spireme. The end (x) is double. × 1800.

Fig. 4. Primary sporocyte showing different stages in tetrad formation. C-ring forms. × 1800.

Fig. 5. Primary sporocyte; portion of nucleus showing stages in tetrad formation. A-double spireme segment; C-ring forms; X-divergent forms. × 2300.

Fig. 6. Various types of tetrad formation; A, B, C, F, G, H, K, L, show the "cross type," D and E the "rod type," I and J the "ring type." × 3600.

Fig. 7. Primary sporocyte with finished tetrads. × 1800.

Fig. 8. Primary sporocyte showing spindle fibres, loss of nuclear membrane, and distribution of the tetrads. × 2300.

Fig. 9. Primary sporocyte section showing fewer tetrads and the arrangement on the spindle fibres. × 2300.

Fig. 10. Primary sporocyte, a prophase showing concentration of the tetrads into the nuclear plate. × 2300.

Fig. 11. Primary sporocyte; metaphase showing completed spindle. C-centrosome. × 2300.

Fig. 12. Primary sporocyte, anaphase showing division of tetrads into dyads. D-dyads. C-centrosome. × 1800.

Fig. 13. Secondary sporocyte; metaphase showing arrangement of elongate dyads in the nuclear plate. C-centrosome. × 1800.

Fig. 14. Late anaphase of the division of the secondary sporocyte showing the four nuclei free in the cell, with connecting spindle fibres. × 2300.

Fig. 15. Early spores. The cell plates have formed; remnants of the spindle fibres can still be seen. × 1800.

Fig. 16. Young spore. Nucleoli have reappeared. × 1800.

Fig. 17. Primary archesporium; a few of the chromosomes during the early stage of division. They are distinctly double. × 2300.

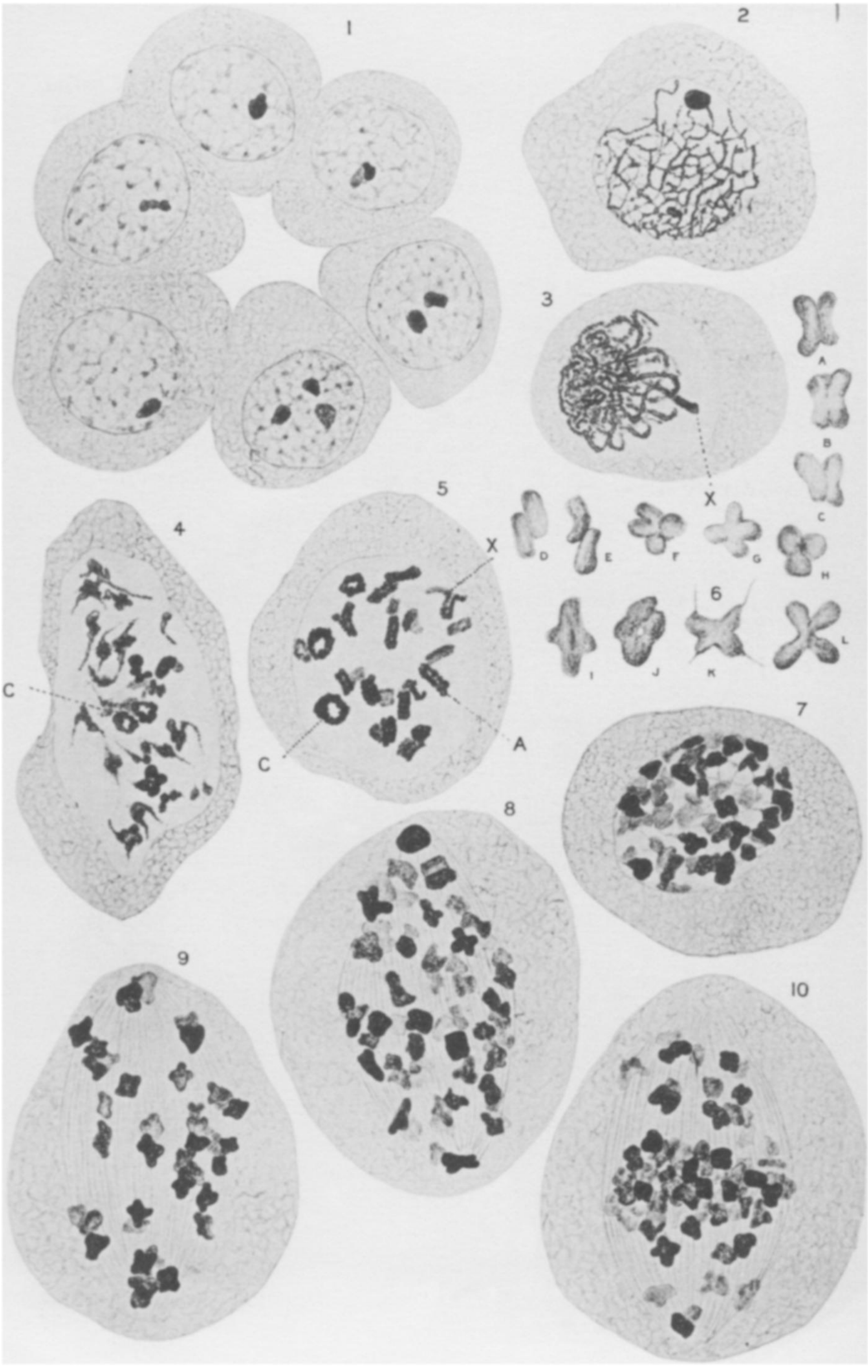
Fig. 18. Somatic cell from a young fern plant, in division. × 2300.

Fig. 19. Primary sporocyte (*Adiantum*) showing formation of tetrads. C-ring form. A-cross form. × 2300.

Fig. 20. Primary sporocyte showing division of tetrads into the nuclear plate. C-ring forms. × 2300.

Fig. 21. Primary sporocyte showing division of the tetrads to form dyads. (D). × 2300.

BULLETIN OF THE TORREY BOTANICAL CLUB. PLATE 295.



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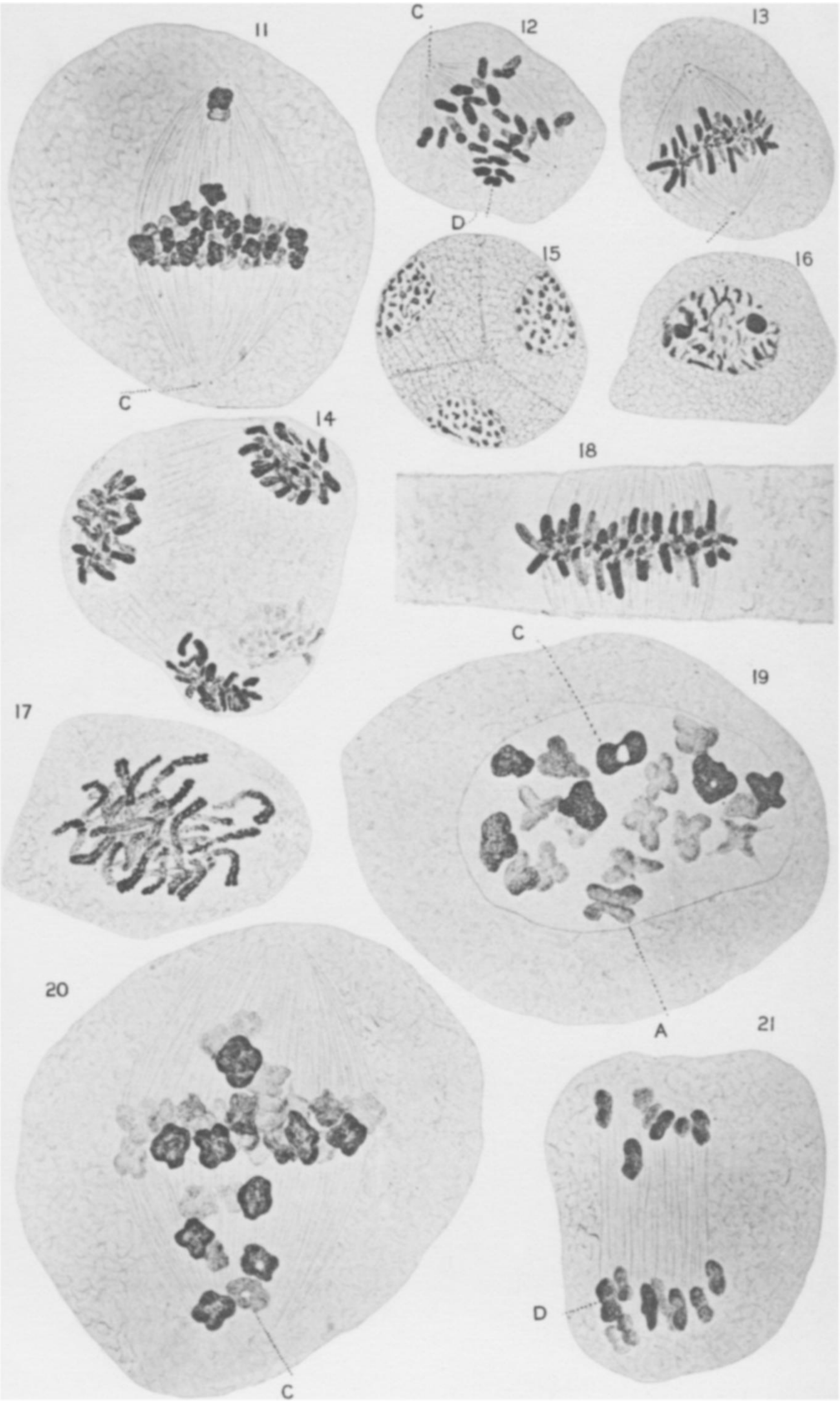
BULLETIN OF THE TORREY BOTANICAL CLUB. PLATE 296.



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